



Year: 2019

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Abstract: Many metastatic melanoma patients experience durable responses to anti-PD1 and/or anti-CTLA4, however, a significant proportion (over 50%) do not benefit from the therapies. In this study, we sought to assess pretreatment liquid biopsies for biomarkers that may correlate with response to checkpoint blockade. We measured the combinatorial diversity evenness of the T-cell receptor (TCR) repertoire (the DE50, with low values corresponding to more clonality and lack of TCR diversity) in pretreatment liquid biopsies from melanoma patients treated with anti-CTLA4 (n = 42) or anti-PD1 (n = 38) using a multi-N-plex PCR assay on genomic DNA (gDNA). A ROC curve determined the optimal threshold for a dichotomized analysis according to objective responses as defined by RECIST1.1. Correlations between treatment outcome, clinical variables, and DE50 were assessed in multivariate regression models and confirmed with Fisher exact tests. In samples obtained prior to treatment initiation, we showed that low DE50 values were predictive of a longer progression-free survival and good responses to PD-1 blockade, but on the other hand predicted a poor responses to CTLA4 inhibition. Multivariate logistic regression models identified DE50 as the only independent predictive factor for response to anti-CTLA4 therapy (P = 0.03) and anti-PD1 therapy (P = 0.001). Fisher exact tests confirmed the association of low DE50 with response in the anti-CTLA4 (P = 0.041) and the anti-PD1 cohort (P = 0.0016). Thus, the evaluation of basal TCR repertoire diversity in peripheral blood, using a PCR-based method, could help predict responses to anti-PD1 and anti-CTLA4 therapies.

DOI: <https://doi.org/10.1158/2326-6066.CIR-18-0136>

Posted at the Zurich Open Repository and Archive, University of Zurich

ZORA URL: <https://doi.org/10.5167/uzh-159305>

Journal Article

Accepted Version

Originally published at:

Hogan, Sabrina A ; Courtier, Anaïs ; Cheng, Phil F ; Jaberg-Bentele, Nicoletta F ; Goldinger, Simone M ; Manuel, Manuarii ; Perez, Solène ; Plantier, Nadia ; Mouret, Jean-François ; Nguyen-Kim, Thi Dan Linh ; Raaijmakers, Marieke I G ; Kvistborg, Pia ; Pasqual, Nicolas ; Haanen, John B A G ; Dummer, Reinhard ; Levesque, Mitchell P (2019). Peripheral blood TCR repertoire profiling may facilitate patient stratification for immunotherapy against melanoma. *Cancer Immunology Research*, 7(1):77-85.

DOI: <https://doi.org/10.1158/2326-6066.CIR-18-0136>

Peripheral blood TCR repertoire profiling may facilitate patient stratification for immunotherapy against melanoma.

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Running Title: TCR repertoire diversity, a biomarker for immunotherapy

KEYWORDS: melanoma, immunotherapy, TCR, biomarker, predictive

Grant support: This work was partially supported by a fellowship to S.A.H. from a European Training Network MELGEN funded consortium, and a research grant from Bristol-Myers Squibb, who were not involved in the experimental design, data analysis or interpretation, or the writing of the manuscript.

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Disclosure of Potential Conflicts of Interest: SMG has intermittent advisory relationship and receives travel grant support by MSD, BMS, Novartis and Roche. She received research support by the University of Zurich and medAlumni.

MPL has previously received research support from BMS, Roche, and Novartis. The former company ImmunID Technologies provided the *ImmunTraCkeR* assays reported here. No potential conflicts of interest were disclosed by the other authors.

Abstract:

Many metastatic melanoma patients experience durable responses to anti-PD1 and/or anti-CTLA4, however, a significant proportion (over 50%) do not benefit from the therapies. In

this study, we sought to assess pretreatment liquid biopsies for biomarkers that may correlate with response to checkpoint blockade. We measured the combinatorial diversity evenness of the T-cell receptor (TCR) repertoire (the DE₅₀, with low values corresponding to more clonality and lack of TCR diversity) in pretreatment liquid biopsies from melanoma patients treated with anti-CTLA4 (n = 42) or anti-PD1 (n = 38) using a multi-N-plex PCR assay on genomic DNA (gDNA). A ROC curve determined the optimal threshold for a dichotomized analysis according to objective responses as defined by RECIST1.1. Correlations between treatment outcome, clinical variables, and DE₅₀ were assessed in multivariate regression models and confirmed with Fisher exact tests. In samples obtained prior to treatment initiation, we showed that low DE₅₀ values were predictive of a longer progression-free survival and good responses to PD-1 blockade, but on the other hand predicted a poor responses to CTLA4 inhibition. Multivariate logistic regression models identified DE₅₀ as the only independent predictive factor for response to anti-CTLA4 therapy ($P = 0.03$) and anti-PD1 therapy ($P = 0.001$). Fisher exact tests confirmed the association of low DE₅₀ with response in the anti-CTLA4 ($P = 0.041$) and the anti-PD1 cohort ($P = 0.0016$). Thus, the evaluation of basal TCR repertoire diversity in peripheral blood, using a PCR-based method, could help predict responses to anti-PD1 and anti-CTLA4 therapies.

Introduction:

Despite durable objective responses (OR) seen with the FDA-approved immune checkpoint inhibitor (ICI) drugs anti-PD1 and anti-CTLA4, more than 50% of metastatic melanoma patients do not respond to these treatments (1). ICI therapies aim to modulate the immune system in order to enhance endogenous T-cell mediated anti-tumoral responses. This process allows patients to mount an immune response against the tumor with limited side effects. Thanks to the advent of those new therapies, in the last 6 years median survival increased from 9 months to 18 months (2). However, compared to previous treatments (i.e. targeted therapies), the mode of action of ICIs often lengthens the time until OR can be assessed. As a result, patients who do not respond to those therapies are potentially exposed to unnecessary side effects, are wasting precious time and may miss their treatment window. Even though few other therapeutic options currently exist, ongoing clinical trials (3-5) may produce a more diverse panel of treatments to choose from in the near future. Thus, a biomarker identifying patients who will respond to anti-PD1 or anti-CTLA4 monotherapies (or combination therapies) would help optimizing patients' stratification in order to achieve the best possible therapeutic outcome given the available drugs. Since tumor-specific T cells present at the tumor site can also be found in the periphery in absence of metastasis (6,7), we hypothesized that the peripheral TCR repertoire could be used to detect clonal expansion of reactive T cells in melanoma patients. We used a PCR-based method to detect every possible V-J rearrangement in the CDR3 region of the TCR β chain in peripheral blood samples from melanoma patients. The aim of this retrospective study was to assess whether the T-cell receptor (TCR) repertoire diversity evenness (DE₅₀) before treatment initiation could predict which subpopulation of patients was more likely to respond to anti-PD1 or anti-CTLA4 therapy. We showed that patients with a low DE₅₀ score did not respond to anti-CTLA4 treatment but did respond to anti-PD1 treatment. Thus, we demonstrated that the quantification of TCR repertoire diversity in the peripheral blood of melanoma patients, prior

to treatment initiation, could represent a predictive biomarker to guide the use of immunotherapies to the most appropriate patients. However, given the small size of our patient cohorts, these results need to be validated on a larger scale as well as in cohorts world-wide before they can be applied in a clinical setting.

Material and Methods

Patients

All experiments were carried out according to the Declaration of Helsinki principles after approval by the ethics committee (Swissethics, Kantonale Ethikkommission Zurich, vote number ZK.Nr.2014-0425). The study was performed on surplus material from two different cohorts of patients after having obtained written, informed consent approved by the local IRB (EK.647/800, KEK-ZH.2014-0425, N03LAM). The first cohort included 42 patients treated with anti-CTLA4 therapy, 18 were treated at the Netherlands Cancer Institute (NKI) and 24 at the University Hospital Zurich (USZ). Patients from the NKI were randomly assigned to the study whereas patients from USZ were selected to enrich the responder ratio. Additional clinical variables were collected around baseline (in a range of 30 days from treatment initiation). To harmonize the values of lactate dehydrogenase (LDH) between the two sites, LDH/upper limit of normal (480 U/l and 250U/l for UZH and NKI, respectively) was calculated. The second cohort was composed of 38 patients treated with anti-PD1 at the USZ. As for the CTLA4 cohort, absolute lymphocyte count (ALC), absolute neutrophil count (ANC), LDH, as well as S100 levels and leukocyte count were documented at baseline (all between -17 and +1 day from treatment start day, except for three LDH and eight S100 concentrations, which were collected between -42 days and + 27 days from the start of treatment when their values were stable over time). The OR was determined by Response Evaluation Criteria in Solid Tumors (RECIST1.1) after 12 weeks of treatment (average = 11.28, median = 11.64, range = 4.7-17.7). Responders were classified as undergoing a

complete response (CR) or a partial response (PR) and non-responders were defined as stable disease (SD) or progressive disease (PD) state. Progression-free survival (PFS) represents the period between the treatment initiation to the date of progression or death, whichever occurred first. Patients' characteristics are shown in Table 1.

ImmunTraCkeR (Multiplex PCR assay)

Peripheral blood mononuclear cell (PBMCs) from melanoma patients, prior to anti-CTLA4 (n = 42) or anti-PD1 (n = 38) treatment, were obtained by Ficoll-gradient centrifugation (Ficoll-Paque Plus, GE healthcare). Genomic DNA was extracted from frozen PBMC samples using High Pure PCR Template Preparation Kit (Roche) and concentrated using Amicon ultra 0.5 mL Centrifugal Filters (Millipore). ImmunTraCkeR[®] (8), a TCR repertoire-profiling tool, was used to study the T-cell immune repertoire diversity based on the detection of V-J rearrangements of the CDR3 region of the TCR β chain, as previously described (9). Briefly, multiplex PCR was performed using upstream primers specific for each V and downstream primer for each J segments; covering all the possible combinations (based on the international ImMunoGeneTics information system[®], IMGT[®], <http://www.imgt.org>). PCR products were separated by microfluidic migration (Labchip GX, Perkin Elmer) using a lab-on-a-chip (HT DNA 12 K LabChip Kit, Perkin Elmer). The PCR signals obtained were compared using Constel'ID[®], a software that generates 3D maps representing the immune status of each patient. The diversity of the TCR pool was studied through the evenness of the repertoire based on frequencies of specific V-J rearrangements. For each patient, a diversity evenness score (DE₅₀) was calculated as follow:

$$DE_{50} = \frac{\text{nb rearrangements accounting for 50\% total map intensity}}{\text{total nb rearrangements present}}$$

A high score corresponds to a homogenous occurrence of each V-J rearrangement, whereas a low score represents a skew in the frequency towards specific rearrangements. Fig.1 shows the outline of the analysis pipeline.

Statistical analysis

The optimal thresholds for each variables were calculated by ROC analysis, using the Youden method (10) (*OptimalCutpoints* package in R).

The relationship between OR and each biological/clinical variables was assessed in both cohorts, The prognostic factors, when available, were included in univariate logistic regression analysis with bias adjustment (Firth's method (11)) using a previously ROC-determined cut-off. Independent predictive variables of response were identified by a logistic regression analysis using a backward selection procedure. The added value of each selected variable in the models was evaluated using a penalized likelihood ratio chi-squared; likelihood scores of the model evaluated with and without the biomarker were compared, considering that lower likelihood scores will indicate better fitting models. The analyses including DE₅₀ in the anti-PD1 cohort were performed using bias adjustment (Firth's method (11)). The association between response and the high vs low DE₅₀ group was assessed by a Fisher exact test.

Relationship between PFS and the available biological/clinical variables was assessed by a univariate Cox proportional hazard regression model using the same previously ROC-determined cut-offs. Independent predictive variables of PFS were identified by a multivariate Cox regression analysis using a backward selection procedure. The added value of each selected variable in the models was evaluated using a likelihood ratio test; likelihood scores of the model evaluated with and without the biomarker were compared, considering that lower likelihood scores will indicate better fitting models. For the Cox regression model assessing the correlation with PFS, NRAS mutation status was excluded from the backward selection

process because these data were missing too many pieces of information, which would limit the power of the test.

Results of the regression models are presented in terms of β -coefficients. β -coefficients describe the change in the natural logarithm of the hazard ratio (HR) or odd ratio (OR) per unit of independent variable. ($e^{\beta_{coeff}} = HR \text{ or } OR$)

Results

Multiplex PCR assay

Diversity of the TCR repertoire was evaluated in a blinded fashion, using a multi-N-plex PCR assay, which measures combinatorial diversity between V and J genes of the β chain at the complementarity determining region 3 (CDR3), where most of the diversity is contained. In order to characterize the diversity of the repertoire, we calculated a diversity evenness score (DE₅₀). The DE₅₀ represents the ratio of the number of rearrangements among the most frequent necessary to account for 50% of the global map intensity (cumulative sum of each rearrangement's frequency) divided by the total number of rearrangements present. A TCR repertoire that is composed of an even frequency distribution of different V-J rearrangements is considered to have low clonality and thus a high DE₅₀ score. A TCR repertoire that is dominated by some specific V-J rearrangements has a low DE₅₀ and is considered to be more clonal.

Patients and treatments

In this retrospective study, baseline frozen PBMC samples from two separate cohorts of patients were analyzed. The first of the two cohorts included 42 metastatic melanoma patients who received ipilimumab at 3 mg/kg every 3 weeks, for a maximum of 4 infusions. Only patients who received at least 2 infusions were included. Absolute lymphocyte count (ALC),

absolute neutrophil count (ANC), and lactate dehydrogenase (LDH) levels were documented at baseline.

The second cohort was composed of 38 patients treated with anti-PD1. 37 patients received pembrolizumab at 2mg/kg (or 10mg/kg for patients participating in the NCT01704287 study) every 3 weeks and 1 patient received nivolumab at 3mg/kg every 2 weeks. Patients who received fewer than 3 infusions were excluded from the analysis. The median follow-up was 11.38 months (mean = 13.08) for patients who died and 32.75 months (median = 36.49) for patients who were still alive at the last follow-up. As for the CTLA4 cohort, ALC, ANC, LDH, as well as S100 levels, basophil, eosinophil and leukocyte counts were documented at baseline. We investigated the potential effect of prior treatment on each of the factors and outcome and found no correlation (Supplementary Fig. S1). Patients' characteristics are shown in Table 1.

The OR was determined by Response Evaluation Criteria in Solid Tumors (RECIST1.1) after at least 12 weeks of treatment (average = 11.28, median = 11.64, range = 4.7-17.7). We evaluated response according to objective response rate, that is complete response (CR) and partial response (PR) are considered to be responders and stable disease (SD) and PD are considered to be non-responders. Survival plots for PFS and overall survival (OS) are shown in Fig. 2.

Analysis of pretreatment clinical parameters and correlation with response.

Firstly, for each clinical parameter, an appropriate threshold for dichotomized analysis was determined by a receiver operating characteristic (ROC) curve. The optimal cutoff points separating responders from non-responders were calculated using the Youden method (10). Thresholds are shown next to the variables in each of the tests.

To assess the predictive potential of DE₅₀ and the different clinical variables on response to treatment, reliable prognostic factors were included in univariate logistic regression analysis

using previously ROC-determined cut-offs (Fig. 3). In the anti-CTLA4 treated cohort, only DE₅₀ appears to be significant in the univariate model, and it is the only independent factor that is conserved in the multivariate model (Fig. 3A). Since the only outcome variable available for all the patients in this cohort was OR, no survival analysis could be performed. In the anti-PD1 treated cohort, at the univariate level, both LDH and DE₅₀ are significantly correlated with response to treatment. To determine if the candidate predictive factors of our model were independent predictors of response, we performed a multivariate logistic regression analysis using a backward selection procedure. The best fitting model only identified DE₅₀ as independent predictive factor (Fig. 3B).

Correlation between baseline DE₅₀ levels and response to treatment

In order to identify the predictive potential of baseline TCR repertoire clonality and its association with clinical benefit, we analyzed the relationship between low and high DE₅₀ and response to treatment (Fig. 4). In the anti-CTLA4 treated cohort, all the patients who had DE₅₀ < 20.03% at baseline did not respond to the treatment ($p = 0.041$) (Fig. 4A). Conversely, in the anti-PD1 treated cohort, a baseline DE₅₀ level below 20.4% was associated with good response to the treatment at 12 weeks ($p = 0.0016$) (Fig. 4B).

Identification of clinical parameters linked with PFS in the anti-PD1 cohort

Independent predictive variables of PFS were identified by a Cox regression analysis using the ROC determined cut-offs. The univariate model identified LDH (β coeff = -0.945, 95%CI = -1.761- -0.129, $P = 0.023$) and S100 (β coeff = -1.814, 95%CI = -3.027- -0.601, $P = 0.003$) as the only two variables significantly correlated with PFS (Fig. 5A). Backward selection procedure was used to extract the most explanatory variable for the multivariate model. The multivariate Cox regression for PFS retained DE₅₀, ANC/ALC, ALC, and S100; only S100 appears not to be an independent factor associated with PFS ($p > 0.05$) (Fig. 5B).

Discussion

The approval of ICIs as first line treatment for melanoma (in 2011) and subsequent developments represent a huge improvement in cancer management. However, patients' responses are still variable and unpredictable. Few reliable biomarkers are available in clinical practice to optimize patient selection and treatment efficacy for immunotherapy.

The first candidate to be examined for anti-PD1 therapy was PD-L1. However, intratumoral PD-L1 expression failed as a predictive biomarker, since PD-L1 negative patients also responded to the treatment (12,13). This result may be explained by the lack of a standardized method of detection (different cut-offs and kits were used) or by the fact that the biopsy used for the assay does not account for the full tumor heterogeneity or the dynamic receptor expression (14). The other drawback inherent in PD-L1 or other tumor staining assay is the need for at least part of the tumor to be sampled, which requires an invasive procedure and may not be feasible due to the location of the tumor. Liquid biopsies derived from blood or serum avoid the problem of invasive procedures. In the context of anti-PD1 treatment, some potential predictive markers have been tested in the peripheral blood, namely, LDH levels as well as eosinophil, monocyte and lymphocyte counts (15,16). In patients treated with anti-CTLA4, studies have investigated lymphocyte, monocyte, eosinophil or neutrophil counts as predictive markers of patients' responses (17-19). The results of those studies are contradictory, and seems to hold more prognostic than predictive information (20,21). Furthermore, serum levels of C-reactive protein, LDH, soluble CD25 and VEGF have been associated with clinical outcome (22-24). Unfortunately, most of the analyses have been performed on small cohorts and none of these potential markers have been validated prospectively (25), except for very high LDH levels which is associated with poor prognosis in anti-CTLA4 treated patients (26). No predictive biomarker for patient stratification prior to

treatment other than routine PD-L1 staining (27) has yet proven robust enough to be used in the clinic.

The efficacy of ICIs relies on the activation and effector functions of the adaptive immune system and largely on T cells. However, the exact mechanisms of action are not understood.

Anti-CTLA4 was shown to lower the threshold for T-cell priming and therefore allow expansion of a higher number of effector T cells (28). Anti-CTLA4 also increases clonal expansion of memory T cells (29) and depletes regulatory T cells (Tregs) in the tumor micro-environment (30) leading to an increase in the effector T-cell/Treg ratio at the tumor site (31).

The major reported effect of PD1 blockade, in the context of cancer treatment, was the restoration of T-cell functions during the effector phase. Anti-PD1 was demonstrated to salvage exhausted CD8⁺ T cells, restoring their cytotoxic potential, leading to the destruction of tumor cells (32). Anti-PD1 was also reported to affect Treg differentiation and function by preventing PD1 ligation to PD-L1 on antigen presenting cells, which have been shown to play a key role in Treg's development (33). However, PD1 has a broad expression pattern: it is expressed on T cells as well as B-cells, natural killer cells, dendritic cells, and myeloid cells. Its ligands, PD-L1 and PD-L2, are also expressed on various hematopoietic and non-hematopoietic cells as well as cancer cells (34,35). As a result, anti-PD1 therapy can affect various cell types and pathways in ways still being investigated (36,37). More research is required to understand the exact modes of anti-CTLA4 and anti-PD1 action. Nevertheless, the effect of ICI therapies on the TCR repertoire has been intensively studied. Several groups have shown that anti-CTLA4 and anti-PD1 therapies reshape the TCR repertoire in different ways (38). Anti-CTLA4 broadens the T-cell repertoire, whereas anti-PD1 drives proliferation of a restricted number of clones, giving rise to a skewed peripheral repertoire .

Anti-CTLA4 expands the peripheral TCR repertoire in a non-specific manner by inhibiting Tregs and increasing the CD8⁺/Treg ratio (39,40). Improved clinical outcome is associated

with reduced clonotype loss and maintenance of high-frequency clonotypes from the start and throughout treatment duration (41). This provides evidence that the baseline TCR repertoire profile might play a role in the response to the treatment.

Investigation of the TCR repertoire in tumors demonstrated a positive association between the clonality of the intra-tumoral T-cell repertoire at baseline and response to anti-PD1 (42). Other groups confirmed those results and showed that anti-PD1 stimulates oligoclonal expansion of certain T-cell clones at the tumor site, in particular those that were present at a higher frequency (43,44). Altogether, these results suggest that the initial shape of the T-cell repertoire may guide therapeutic decisions. Other studies have analyzed the TCR repertoire using next-generation sequencing (NGS), however NGS approaches are currently too expensive and the data processing too time consuming for routine clinical use.

Here we have aimed to develop a tool that could be useful in the clinic for routine patient stratification. The primary objective of this study was to determine whether baseline TCR repertoire diversity could be used as a predictive biomarker of response to anti-PD1 and anti-CTLA4 and how it compares to other predictive variables described in the literature.

In order to reduce the number of factors included in our analysis, we first confirmed that the previous treatments received by the patients did not affect the clinical variables and was not correlated with response (Supplementary Fig. S1). Then, to compare our predictor's performance to other proposed markers in the literature, we performed regression analyses including other relevant factors whenever available. At the multivariate level, using the ROC determined threshold, DE₅₀ was the only significant predictor of response in our cohorts. We also observed that in the univariate analyses, LDH level was significantly correlated with PFS and response to anti-PD1 therapy. Baseline LDH levels are thought to be indicative of future outcome irrespective of treatment (45-47), but can lead to false positive predictions of response. For instance, some patients with high baseline LDH levels can also achieve a good response to anti-PD1 (48) and high and low LDH groups show no difference in treatment

efficacy (49). This underscores the need to find better early predictors of response to ICI therapies.

The only other factors able to independently predict outcome in one of our cohort were the neutrophil-to-lymphocyte ratio (ANC/ALC) and the ALC, which had PFS predictive power in anti-PD1 treated patients. ANC/ALC has already been shown to be a predictive marker for response in anti-PD1 treated melanoma patients (50). Because ANC/ALC is described as a prognostic factor which is also correlated with leukocyte/lymphocyte ratio (51), we investigated this potential association in our cohort (Supplementary Fig. S2). Indeed, we found a similar correlation. Patients with low ANC/ALC expression have more T-cell tumor infiltration compared to the high ANC/ALC group (51). This would support our results since T-cell infiltration is a key element for an appropriate immune response against the tumor. Nevertheless, other studies found a correlation between ANC/ALC and patient outcome regardless of the treatment (52,53).

Overall, our analysis showed that $ALC \geq 1.27$ G/l and $ANC/ALC < 2.86$ are correlated with longer PFS and are also associated with a high lymphocyte to leukocyte ratio. We showed that the detection of a basal clonal TCR repertoire characterized by $DE_{50} < 20.4\%$ in peripheral blood, via a conventional PCR based method, is able to predict response to anti-PD1 treatment at 12 weeks. On the other hand, a clonal TCR repertoire prior to anti-CTLA4 therapy characterized by $DE_{50} < 20.03\%$ is associated with poor response to the treatment. In line with our results, DE_{50} is also significantly correlated with PFS at 12 months (Supplementary Fig. S3A) and OS at 24 months (Supplementary Fig. S3B) in the anti-PD1 cohort of patients.

We have demonstrated that TCR repertoire clonality, represented by the TCR diversity index DE_{50} , can help predict patient outcome under anti-PD1 and anti-CTLA4 therapy. A low DE_{50}

(i.e. clonal repertoire) was associated with a poor response to anti-CTLA4 and with a better response and a longer PFS in patients treated with anti-PD1, which is consistent with previously published literature (9,54).

Our results indicate that patients with a repertoire dominated by specific rearrangements may benefit more from anti-PD1. However, our analysis does not allow us to determine the exact composition of the TCR repertoire in terms of cell types nor to discriminate between pro-tumorigenic (i.e. CD4⁺ Treg) and anti-tumorigenic cell types (i.e. cytotoxic CD8⁺). Yet, two hypothesis would support the rationale of our finding. Firstly, in patients whose immune system fails to eliminate the tumor, there is an increase in exhausted T cells (55). Those cells could correspond to the clonal expansion we detect at baseline and explain the subsequent efficient response to anti-PD1 treatment, which restores function to these tumor-specific T cells. Secondly, baseline T-cell repertoire of non-responders have a higher proportion of CD8⁺ naïve T cells, whereas responders have a larger population of central memory T cells (16). Hence, the higher clonality of the T-cell repertoire seen at baseline could also be due to a higher population of memory T cells, which respond particularly well to PD1 blockade (56).

A clonally expanded TCR repertoire at baseline appears to preclude the efficacy of anti-CTLA4 treatment. A skewed TCR repertoire is the representation of an immune system that is responding to a foreign invasion (57). If, prior to therapy initiation, the patient's immune system has already mounted a response against specific antigens (here, we assume melanoma antigens), treatment with anti-CTLA4 will be of little use. Indeed, the therapy is mainly aimed at eliciting a T-cell response by sequestering the receptor responsible for inhibition. This means that the treatment has little effect on already activated and expanded T-cell populations (58). If the T-cell clones present before therapy initiation were not able to keep the disease under control, anti-CTLA4 treatment will not improve the outcome. Furthermore, a highly clonal repertoire with no response is most likely associated with immune checkpoints up-

regulation and T-cell exhaustion. In keeping with this assumption, anti-CTLA4 based treatment is ineffective on melanoma with high PD-L1 expression (40).

Our data need to be further validated in a larger and independent cohort with similar inclusion criteria. Moreover, additional studies are necessary to assess the TCR specificity of the clonally expanded T cells in responding patients in order to determine which epitopes are being recognized. A limitation of our study is that we cannot demonstrate if the expanded clones that were identified in the responding patients were tumor specific. However, the fact that responders to anti-PD1 therapy showed substantial expansion of only a limited number of clones (Supplementary Fig. S4) would corroborate previous studies demonstrating that the anti-tumor response only relies on a restricted number of specific TCR (59,60). In summary, with this study we propose the analysis of peripheral blood TCR repertoire as a predictive tool to facilitate patient stratification in the context of ICI therapy for melanoma.

Acknowledgments

We thank the biobank in the University Research Priority Program (URPP) for translational cancer research at the University of Zurich for access to the surplus liquid biopsies, and specifically to Melanie Maudrich, Alice Langer, and Tabea Koch for their technical support for this project. S.A.H. was supported by a fellowship from the European Training network MelGen.

References

1. Ascierto PA, Marincola FM. 2015: The Year of Anti-PD-1/PD-L1s Against Melanoma and Beyond. *EBioMedicine* **2015**;2(2):92-3 doi 10.1016/j.ebiom.2015.01.011.
2. Luke JJ, Flaherty KT, Ribas A, Long GV. Targeted agents and immunotherapies: optimizing outcomes in melanoma. *Nat Rev Clin Oncol* **2017**;14(8):463-82 doi 10.1038/nrclinonc.2017.43.
3. Ott PA, Pavlick AC, Johnson DB, Hart LL, Infante JR, Luke JJ, *et al.* A phase II study of glembatumumab vedotin (GV), an antibody-drug conjugate (ADC) targeting gpNMB, in advanced melanoma. *J Clin Oncol* **2017**;35 doi 10.1200/JCO.2017.35.15_suppl.109.
4. Danielli R, Patuzzo R, Di Giacomo AM, Gallino G, Maurichi A, Di Florio A, *et al.* Intralesional administration of L19-IL2/L19-TNF in stage III or stage IVM1a melanoma patients: results of a phase II study. *Cancer Immunol Immunother* **2015**;64(8):999-1009 doi 10.1007/s00262-015-1704-6.
5. Ascierto PA, Melero I, Bhatia S, Bono P, Sanborn RE, Lipson EJ. Initial efficacy of anti-lymphocyte activation gene-3 (anti-LAG-3; BMS-986016) in combination with nivolumab (nivo) in pts with melanoma (MEL) previously treated with anti-PD-1/PD-L1 therapy. *J Clin Oncol* **2017**: 9520- doi 10.1200/JCO.2017.35.15_suppl.9520.
6. Cohen CJ, Gartner JJ, Horovitz-Fried M, Shamalov K, Trebska-McGowan K, Bliskovsky VV, *et al.* Isolation of neoantigen-specific T cells from tumor and peripheral lymphocytes. *J Clin Invest* **2015**;125(10):3981-91 doi 10.1172/JCI82416.
7. Gros A, Parkhurst MR, Tran E, Pasetto A, Robbins PF, Ilyas S, *et al.* Prospective identification of neoantigen-specific lymphocytes in the peripheral blood of melanoma patients. *Nat Med* **2016**;22(4):433-8 doi 10.1038/nm.4051.
8. Tanneau I, Nondé A, Courtier A, Parmentier G, Noël M, Grivès A, *et al.* ImmunTraCkeR® as a reliable TCR repertoire profiling tool to understand immune response and to explore immunotherapy biomarkers. *Journal for ImmunoTherapy of Cancer* **2013**;1(Suppl 1):P112 doi 10.1186/2051-1426-1-s1-p112.
9. Postow MA, Manuel M, Wong P, Yuan J, Dong Z, Liu C, *et al.* Peripheral T cell receptor diversity is associated with clinical outcomes following ipilimumab treatment in metastatic melanoma. *J Immunother Cancer* **2015**;3:23 doi 10.1186/s40425-015-0070-4.
10. Youden WJ. Index for rating diagnostic tests. *Cancer* **1950**;3(1):32-5.
11. Wang X. Firth logistic regression for rare variant association tests. *Front Genet* **2014**;5:187 doi 10.3389/fgene.2014.00187.
12. Weber JS, D'Angelo SP, Minor D, Hodi FS, Gutzmer R, Neyns B, *et al.* Nivolumab versus chemotherapy in patients with advanced melanoma who progressed after anti-CTLA-4 treatment (CheckMate 037): a randomised, controlled, open-label, phase 3 trial. *The Lancet Oncology* **2015**;16(4):375-84 doi 10.1016/s1470-2045(15)70076-8.
13. Robert C, Long GV, Brady B, Dutriaux C, Maio M, Mortier L, *et al.* Nivolumab in previously untreated melanoma without BRAF mutation. *N Engl J Med* **2015**;372(4):320-30 doi 10.1056/NEJMoa1412082.
14. Patel SP, Kurzrock R. PD-L1 Expression as a Predictive Biomarker in Cancer Immunotherapy. *Molecular cancer therapeutics* **2015**;14(4):847-56 doi 10.1158/1535-7163.mct-14-0983
- 10.1158/1535-7163.MCT-14-0983. Epub 2015 Feb 18.
15. Weide B, Martens A, Hassel JC, Berking C, Postow MA, Bisschop K, *et al.* Baseline Biomarkers for Outcome of Melanoma Patients Treated with Pembrolizumab. *Clin Cancer Res* **2016**;22(22):5487-96 doi 10.1158/1078-0432.CCR-16-0127.
16. Krieg C, Nowicka M, Guglietta S, Schindler S, Hartmann FJ, Weber LM, *et al.* High-dimensional single-cell analysis predicts response to anti-PD-1 immunotherapy. *Nat Med* **2018**;24(2):144-53 doi 10.1038/nm.4466.

17. Ferrucci PF, Ascierto PA, Pigozzo J, Del Vecchio M, Maio M, Cappellini GCA, *et al.* Baseline neutrophils and derived neutrophil-to-lymphocyte ratio: prognostic relevance in metastatic melanoma patients receiving ipilimumab. *Ann Oncol* **2016**;27(4):732-8 doi 10.1093/annonc/mdw016.
18. Alexander M, Mellor JD, McArthur G, Kee D. Ipilimumab in pretreated patients with unresectable or metastatic cutaneous, uveal and mucosal melanoma. *Med J Aust* **2014**;201(1):49-53 doi 10.5694/mja13.10448.
19. Martens A, Wistuba-Hamprecht K, Geukes Foppen MH, Yuan J, Postow MA, Wong P, *et al.* Baseline peripheral blood biomarkers associated with clinical outcome of advanced melanoma patients treated with ipilimumab. *Clin Cancer Res* **2016** doi 10.1158/1078-0432.ccr-15-2412.
20. Maleki Vareki S, Garrigos C, Duran I. Biomarkers of response to PD-1/PD-L1 inhibition. *Crit Rev Oncol Hematol* **2017**;116:116-24 doi 10.1016/j.critrevonc.2017.06.001.
21. Postow MA, Chasalow SD, Yuan JD, Kuk D, Panageas KS, Cheng M, *et al.* Pharmacodynamic effect of ipilimumab on absolute lymphocyte count (ALC) and association with overall survival in patients with advanced melanoma. *J Clin Oncol* **2013**;31(15) doi 10.1200/jco.2013.31.15_suppl.9052.
22. Yuan JD, Zhou J, Dong ZW, Tandon S, Kuk D, Panageas KS, *et al.* Pretreatment Serum VEGF Is Associated with Clinical Response and Overall Survival in Advanced Melanoma Patients Treated with Ipilimumab. *Cancer Immunol Res* **2014**;2(2):127-32 doi 10.1158/2326-6066.Cir-13-0163.
23. Hannani D, Vetizou M, Enot D, Rusakiewicz S, Chaput N, Klatzmann D, *et al.* Anticancer immunotherapy by CTLA-4 blockade: obligatory contribution of IL-2 receptors and negative prognostic impact of soluble CD25. *Cell Res* **2015**;25(2):208-24 doi 10.1038/cr.2015.3.
24. Kelderman S, Heemskerk B, van Tinteren H, van den Brom RR, Hospers GA, van den Eertwegh AJ, *et al.* Lactate dehydrogenase as a selection criterion for ipilimumab treatment in metastatic melanoma. *Cancer Immunol Immunother* **2014**;63(5):449-58 doi 10.1007/s00262-014-1528-9.
25. Yuan J, Hegde PS, Clynes R, Foukas PG, Harari A, Kleen TO, *et al.* Novel technologies and emerging biomarkers for personalized cancer immunotherapy. *J Immunother Cancer* **2016**;4:3 doi 10.1186/s40425-016-0107-3.
26. Wolchok JD, Chiarion-Sileni V, Gonzalez R, Rutkowski P, Grob JJ, Cowey CL, *et al.* Updated results from a phase III trial of nivolumab (NIVO) combined with ipilimumab (IPI) in treatment-naïve patients (pts) with advanced melanoma (MEL) (CheckMate 067). *J Clin Oncol* **2016**;34(15) doi 10.1200/JCO.2016.34.15_suppl.9505.
27. Daud AI, Wolchok JD, Robert C, Hwu WJ, Weber JS, Ribas A, *et al.* Programmed Death-Ligand 1 Expression and Response to the Anti-Programmed Death 1 Antibody Pembrolizumab in Melanoma. *J Clin Oncol* **2016**;34(34):4102-9 doi 10.1200/JCO.2016.67.2477.
28. Gajewski TF, Fallarino F, Fields PE, Rivas F, Alegre ML. Absence of CTLA-4 Lowers the Activation Threshold of Primed CD8+ TCR-Transgenic T Cells: Lack of Correlation with Src Homology Domain 2-Containing Protein Tyrosine Phosphatase. *The Journal of Immunology* **2001**;166(6):3900-7 doi 10.4049/jimmunol.166.6.3900.
29. Pedicord VA, Montalvo W, Leiner IM, Allison JP. Single dose of anti-CTLA-4 enhances CD8+ T-cell memory formation, function, and maintenance. *Proc Natl Acad Sci U S A* **2011**;108(1):266-71 doi 10.1073/pnas.1016791108.
30. Read S, Greenwald R, Izcue A, Robinson N, Mandelbrot D, Francisco L, *et al.* Blockade of CTLA-4 on CD4+CD25+ Regulatory T Cells Abrogates Their Function In Vivo. *The Journal of Immunology* **2006**;177(7):4376-83 doi 10.4049/jimmunol.177.7.4376.
31. Quezada SA, Peggs KS, Curran MA, Allison JP. CTLA4 blockade and GM-CSF combination immunotherapy alters the intratumor balance of effector and regulatory T cells. *J Clin Invest* **2006**;116(7):1935-45 doi 10.1172/JCI27745.
32. Lee J, Ahn E, Kissick HT, Ahmed R. Reinvigorating Exhausted T Cells by Blockade of the PD-1 Pathway. *For Immunopathol Dis Therap* **2015**;6(1-2):7-17 doi 10.1615/ForumImmunDisTher.2015014188.
33. Francisco LM, Salinas VH, Brown KE, Vanguri VK, Freeman GJ, Kuchroo VK, *et al.* PD-L1 regulates the development, maintenance, and function of induced regulatory T cells. *J Exp Med* **2009**;206(13):3015-29 doi 10.1084/jem.20090847.
34. Nguyen LT, Ohashi PS. Clinical blockade of PD1 and LAG3--potential mechanisms of action. *Nature reviews Immunology* **2015**;15(1):45-56 doi 10.1038/nri3790.

35. Messal N, Serriari NE, Pastor S, Nunes JA, Olive D. PD-L2 is expressed on activated human T cells and regulates their function. *Molecular immunology* **2011**;48(15-16):2214-9 doi 10.1016/j.molimm.2011.06.436.
36. Ramelyte E, Schindler SA, Dummer R. The safety of anti PD-1 therapeutics for the treatment of melanoma. *Expert Opin Drug Saf* **2017**;16(1):41-53 doi 10.1080/14740338.2016.1248402.
37. Iwai Y, Hamanishi J, Chamoto K, Honjo T. Cancer immunotherapies targeting the PD-1 signaling pathway. *J Biomed Sci* **2017**;24(1):26 doi 10.1186/s12929-017-0329-9.
38. Parry RV, Chemnitz JM, Frauwirth KA, Lanfranco AR, Braunstein I, Kobayashi SV, *et al.* CTLA-4 and PD-1 receptors inhibit T-cell activation by distinct mechanisms. *Molecular and cellular biology* **2005**;25(21):9543-53 doi 10.1128/MCB.25.21.9543-9553.2005.
39. Robert L, Tsoi J, Wang X, Emerson R, Homet B, Chodon T, *et al.* CTLA4 blockade broadens the peripheral T-cell receptor repertoire. *Clin Cancer Res* **2014**;20(9):2424-32 doi 10.1158/1078-0432.ccr-13-2648.
40. Twyman-Saint Victor C, Rech AJ, Maity A, Rengan R, Pauken KE, Stelekati E, *et al.* Radiation and dual checkpoint blockade activate non-redundant immune mechanisms in cancer. *Nature* **2015**;520(7547):373-7 doi 10.1038/nature14292.
41. Cha E, Klinger M, Hou Y, Cummings C, Ribas A, Faham M, *et al.* Improved survival with T cell clonotype stability after anti-CTLA-4 treatment in cancer patients. *Sci Transl Med* **2014**;6(238):238ra70 doi 10.1126/scitranslmed.3008211.
42. Tumeh PC, Harview CL, Yearley JH, Shintaku IP, Taylor EJ, Robert L, *et al.* PD-1 blockade induces responses by inhibiting adaptive immune resistance. *Nature* **2014**;515(7528):568-71 doi 10.1038/nature13954.
43. Roh W, Chen PL, Reuben A, Spencer CN, Prieto PA, Miller JP, *et al.* Integrated molecular analysis of tumor biopsies on sequential CTLA-4 and PD-1 blockade reveals markers of response and resistance. *Sci Transl Med* **2017**;9(379) doi 10.1126/scitranslmed.aah3560.
44. Inoue H, Park JH, Kiyotani K, Zewde M, Miyashita A, Jinnin M, *et al.* Intratumoral expression levels of PD-L1, GZMA, and HLA-A along with oligoclonal T cell expansion associate with response to nivolumab in metastatic melanoma. *Oncoimmunology* **2016**;5(9):e1204507 doi 10.1080/2162402X.2016.1204507.
45. Balch CM, Gershenwald JE, Soong SJ, Thompson JF, Atkins MB, Byrd DR, *et al.* Final version of 2009 AJCC melanoma staging and classification. *J Clin Oncol* **2009**;27(36):6199-206 doi 10.1200/jco.2009.23.4799
- 10.1200/JCO.2009.23.4799. Epub 2009 Nov 16.
46. Weide B, Richter S, Buttner P, Leiter U, Forschner A, Bauer J, *et al.* Serum S100B, lactate dehydrogenase and brain metastasis are prognostic factors in patients with distant melanoma metastasis and systemic therapy. *PLoS One* **2013**;8(11):e81624 doi 10.1371/journal.pone.0081624.
47. Diem S, Kasenda B, Spain L, Martin-Liberal J, Marconcini R, Gore M, *et al.* Serum lactate dehydrogenase as an early marker for outcome in patients treated with anti-PD-1 therapy in metastatic melanoma. *Br J Cancer* **2016**;114(3):256-61 doi 10.1038/bjc.2015.467.
48. Long GV, Blank C, Ribas A, Mortier L, Carlino MS, Lotem M, *et al.* Impact of baseline serum lactate dehydrogenase concentration on the efficacy of pembrolizumab and ipilimumab in patients with advanced melanoma: data from KEYNOTE-006. *European Journal of Cancer* **2017**;72:S122-S3 doi 10.1016/s0959-8049(17)30482-3.
49. Ribas A, Puzanov I, Dummer R, Schadendorf D, Hamid O, Robert C, *et al.* Pembrolizumab versus investigator-choice chemotherapy for ipilimumab-refractory melanoma (KEYNOTE-002): a randomised, controlled, phase 2 trial. *The Lancet Oncology* **2015**;16(8):908-18 doi 10.1016/s1470-2045(15)00083-2.
50. Fujisawa Y, Yoshino K, Otsuka A, Funakoshi T, Fujimura T, Yamamoto Y, *et al.* Baseline neutrophil to lymphocyte ratio combined with serum LDH level associated with outcome of nivolumab immunotherapy in a Japanese advanced melanoma population. *Br J Dermatol* **2018** doi 10.1111/bjd.16427.
51. Lin G, Liu Y, Li S, Mao Y, Wang J, Shuang Z, *et al.* Elevated neutrophil-to-lymphocyte ratio is an independent poor prognostic factor in patients with intrahepatic cholangiocarcinoma. *Oncotarget* **2016**;7(32):50963-71 doi 10.18632/oncotarget.7680.
52. Blackley E, Lim LE, Moore M, Voskoboynik M, McLean C, Haydon A. Neutrophil to lymphocyte ratio (NLR) as an independent prognostic measure in patients receiving targeted therapy or immunotherapy for stage IV melanoma. 2017 September 8-12, 2017; Madrid.
53. Wei B, Yao M, Xing C, Wang W, Yao J, Hong Y, *et al.* The neutrophil lymphocyte ratio is associated with breast cancer prognosis: an updated systematic review and meta-analysis. *Onco Targets Ther* **2016**;9:5567-75 doi 10.2147/OTT.S108419.

54. Postow M, Manuel M, Wong P, Yuan J, Noel M, Courtier A, *et al.* T cell receptor diversity evaluation to predict patient response to Ipilimumab in metastatic melanoma. *Journal for ImmunoTherapy of Cancer* **2014**;2(Suppl 3):O8 doi 10.1186/2051-1426-2-s3-o8.
55. Jiang Y, Li Y, Zhu B. T-cell exhaustion in the tumor microenvironment. *Cell Death Dis* **2015**;6:e1792 doi 10.1038/cddis.2015.162.
56. Blake SJ, Ching AL, Kenna TJ, Galea R, Large J, Yagita H, *et al.* Blockade of PD-1/PD-L1 promotes adoptive T-cell immunotherapy in a tolerogenic environment. *PLoS One* **2015**;10(3):e0119483 doi 10.1371/journal.pone.0119483.
57. Burnet FM. A Modification of Jerne's Theory of Antibody Production using the Concept of Clonal Selection. *CA: A Cancer Journal for Clinicians* **1976**;26(2):119-21 doi 10.3322/canjclin.26.2.119.
58. Kvistborg P, Philips D, Kelderman S, Hageman L, Ottensmeier C, Joseph-Pietras D, *et al.* Anti-CTLA-4 therapy broadens the melanoma-reactive CD8+ T cell response. *Sci Transl Med* **2014**;6(254):254ra128 doi 10.1126/scitranslmed.3008918.
59. Manne J, Mastrangelo MJ, Sato T, Berd D. TCR rearrangement in lymphocytes infiltrating melanoma metastases after administration of autologous dinitrophenyl-modified vaccine. *J Immunol* **2002**;169(6):3407-12.
60. Willhauck M, Scheibenbogen C, Pawlita M, Möhler T, Thiel E, Keilholz U. Restricted T-cell receptor repertoire in melanoma metastases regressing after cytokine therapy. *Cancer Res* **2003**;63(13):3483-5.

Table 1. Patient characteristics for anti-CTLA4 and anti_PD1 cohorts. N = number of patients in each category. % = representation in percent for each category.

PATIENT CHARACTERISTICS	Anti-PD1		Anti-CTLA4	
	N	%	N	%
Age				
< 40 years old	2	5.3%	3	7.1%
40-55 years old	11	28.9%	14	33.3%
56-70 years old	14	36.8%	18	42.9%
> 70 years old	11	28.9%	7	16.7%
Gender				
Male	27	71.1%	23	54.8%
Female	11	28.9%	19	45.2%
Progression-free survival (PFS)				
< 3 months	9	23.7%	13	59.1%
3-9 months	11	28.9%	6	27.3%
> 9 months	18	47.4%	3	13.6%
Recist 1.1 at ~12 weeks				
CR	1	2.6%	2	4.8%
PR	11	28.9%	10	23.8%
SD	12	31.6%	3	7.1%
PD	14	36.8%	27	64.3%
BRAF mutation status				
Wild type	27	73.0%		
Mutated	10	27.0%		
NRAS mutation status				
Wild type	21	60.0%		
Mutated	14	40.0%		
Previous treatments				
Chemotherapy	3	7.9%		
Targeted therapy	2	5.3%		
Immunotherapy (ipilimumab)	28	73.7%		

Radiotherapy	4	10.5%		
Interferon	1	2.6%		
Lactate dehydrogenase (LDH/ULN)				
< 1	20	54.1%	27	67.5%
≥ 1	17	45.9%	13	32.5%
S100				
< 0.2 µg/l	10	27.8%		
≥ 0.2 µg/l	26	72.2%		
Absolute Neutrophil Count (ANC)				
< 8 G/l	35	92.1%	40	95.2%
≥ 8 G/l	3	7.9%	2	4.8%
Absolute Lymphocyte Count (ALC)				
< 1.5 G/l	25	65.8%	28	66.7%
≥ 1.5 G/l	13	34.2%	14	33.3%
Leukocytes				
< 9.6 G/l	34	89.5%		
≥ 9.6 G/l	4	10.5%		
Basophils				
< 0.15 G/l	20	52.6%		
≥ 0.15 G/l	18	47.4%		
Eosinophils				
< 0.7 G/l	36	94.7%		
≥ 0.7 G/l	2	5.3%		

Figure legends

Fig. 1 Outline of analysis pipeline

Fig. 2 Kaplan-Meier plots showing survival in R (responders) and NR (non-responders) to anti-PD1 treatment. Colored bands represent 95% confidence interval. **A)** PFS probability, **B)** OS probability.

Fig. 3 Logistic regression model assessing the relationship between pretreatment clinical parameters (including DE₅₀) and response to therapy. Forest plots and tables represent the β coefficients of the clinical variables that may affect response to treatment. Blue lines

represent the 95% confidence interval. **A)** Univariate analysis for the anti-CTLA4 treated cohort. * : DE_{50} is the only factor conserved in the multivariate analysis. **B)** Univariate analysis for the anti-PD1 treated cohort. * : DE_{50} is the only factor conserved in the multivariate analysis. In the multivariate analyses, factors were selected using a backward selection process.

Fig. 4 TCR diversity evenness at baseline, prior to treatment. **A)** Dot plot showing DE_{50} at baseline in patients who eventually responded or did not respond to anti-CTLA4 therapy. N= 42. Dashed line = 20.03%. **B)** Dot plot showing DE_{50} levels at baseline in patients who eventually responded or did not respond to anti-PD1 therapy. N= 38. Dashed line = 20.4%. P value calculated with Fisher exact test.

Fig. 5 Cox regression model assessing correlation of clinical variables with PFS in the anti-PD1 cohort. Forest plots and tables represent the β coefficients of the clinical variables that may affect PFS. Blue lines represent the 95% confidence interval. **A)** Univariate analysis. **B)** Multivariate analysis using backward selection procedure.

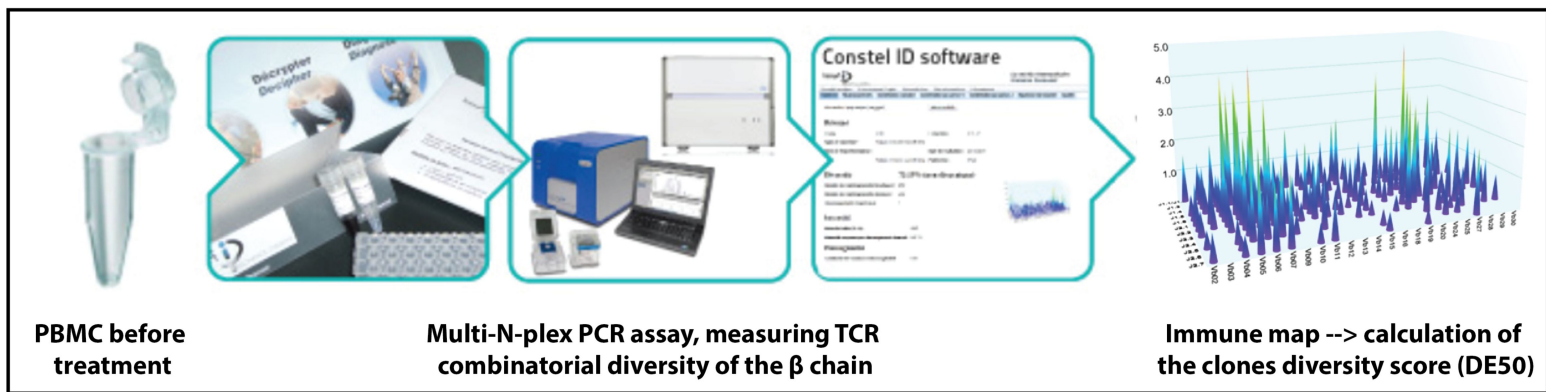
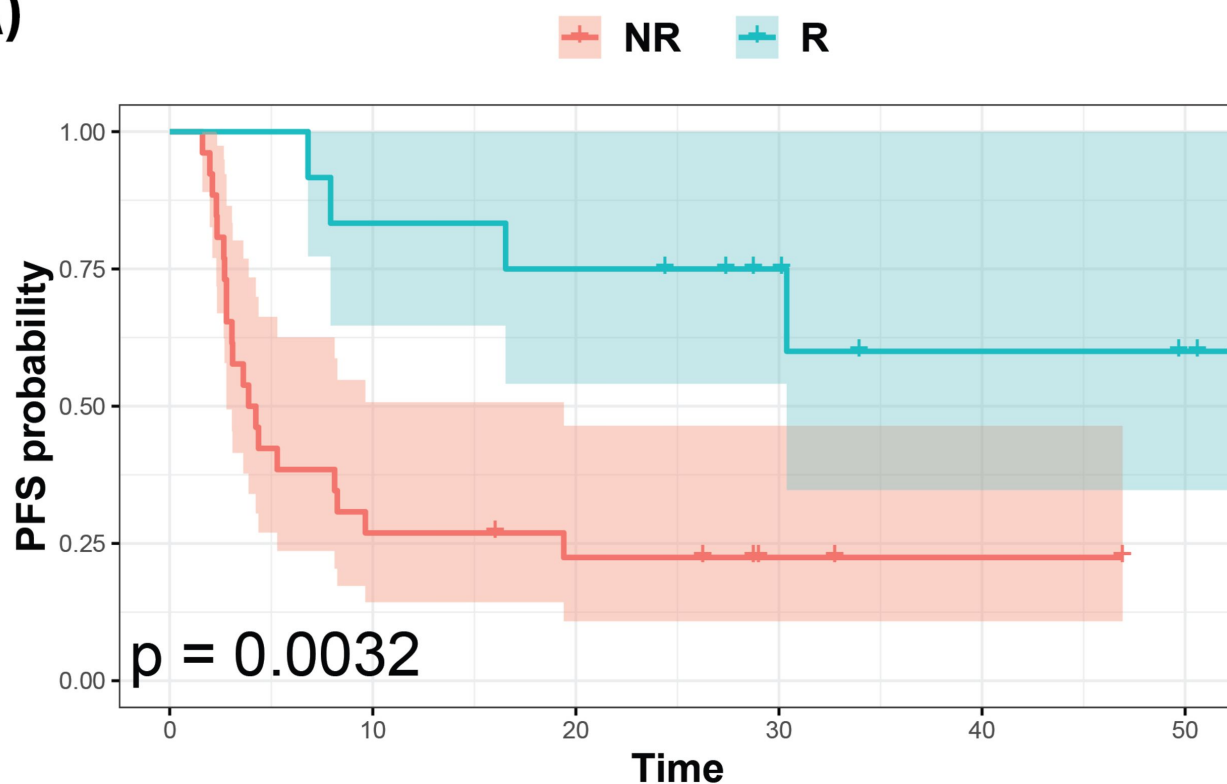


Figure 1

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A)



B)

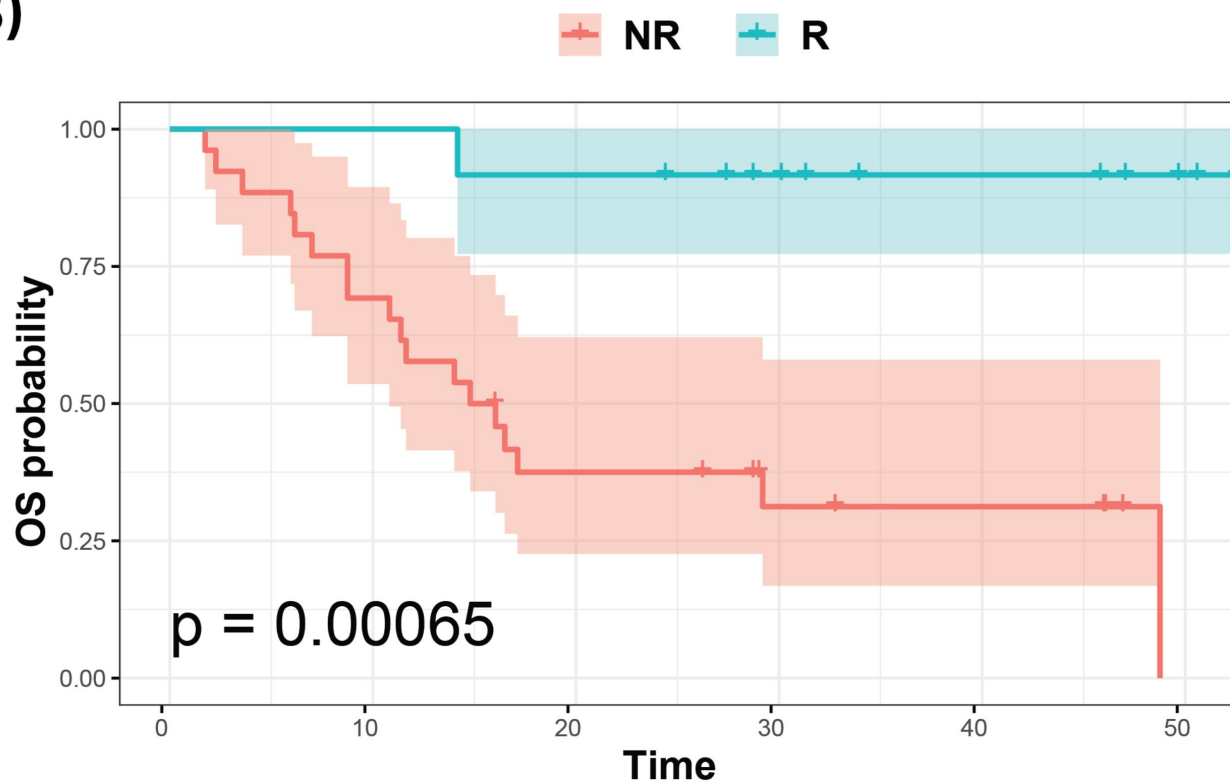


Figure 2

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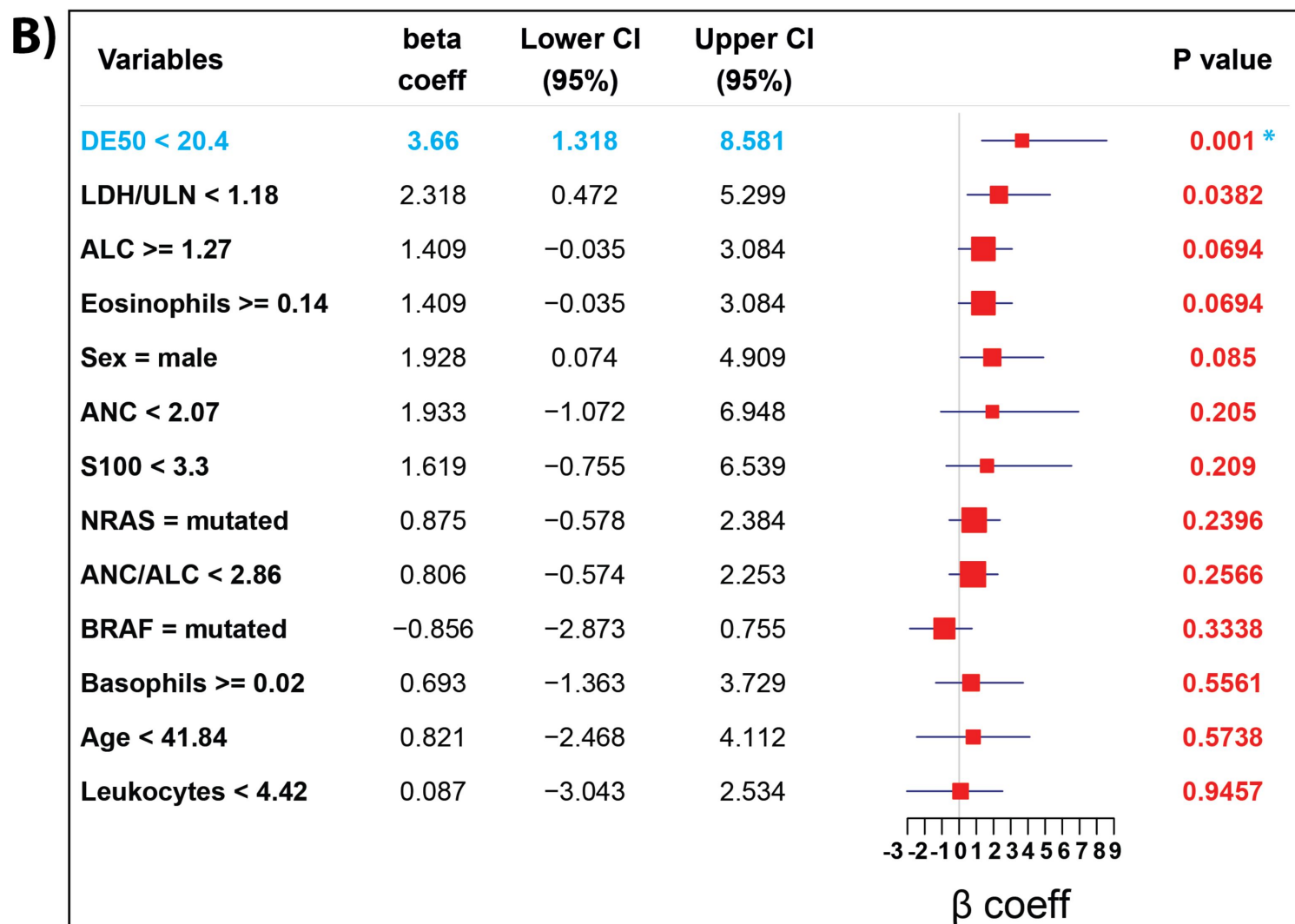
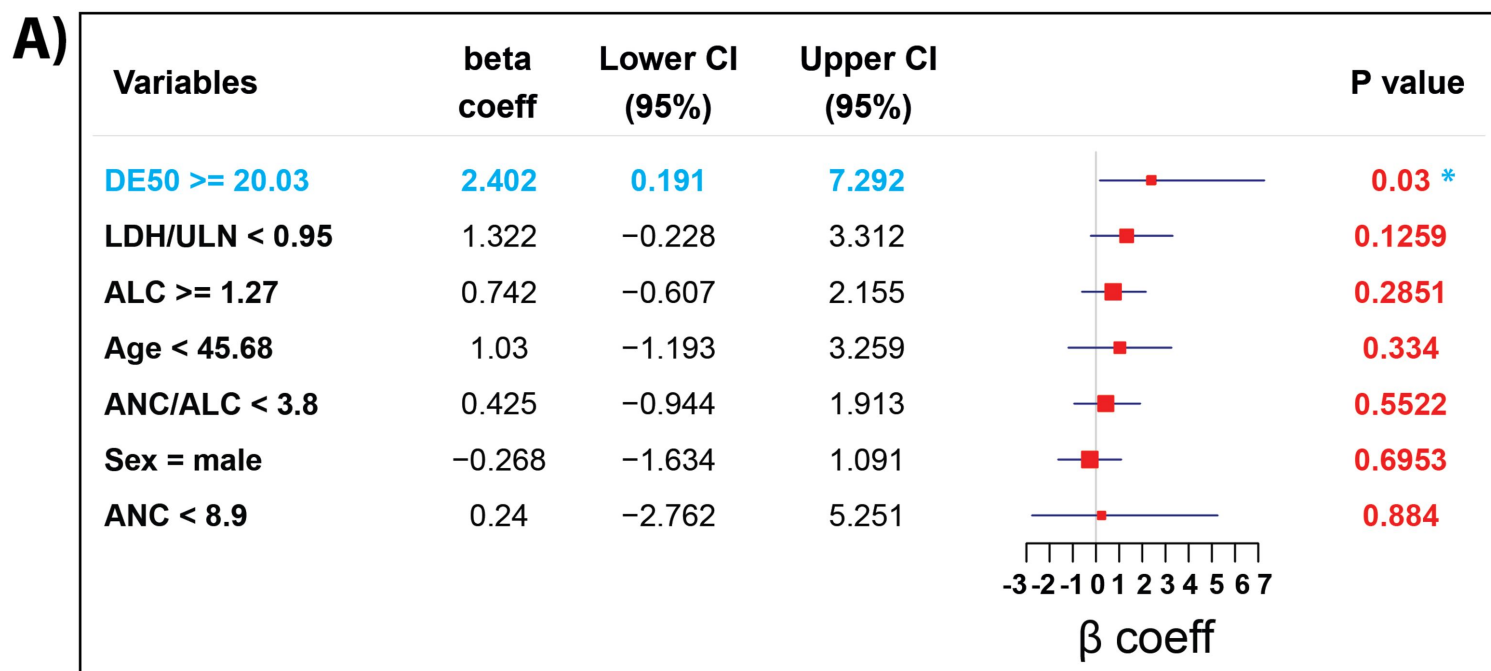


Figure 3

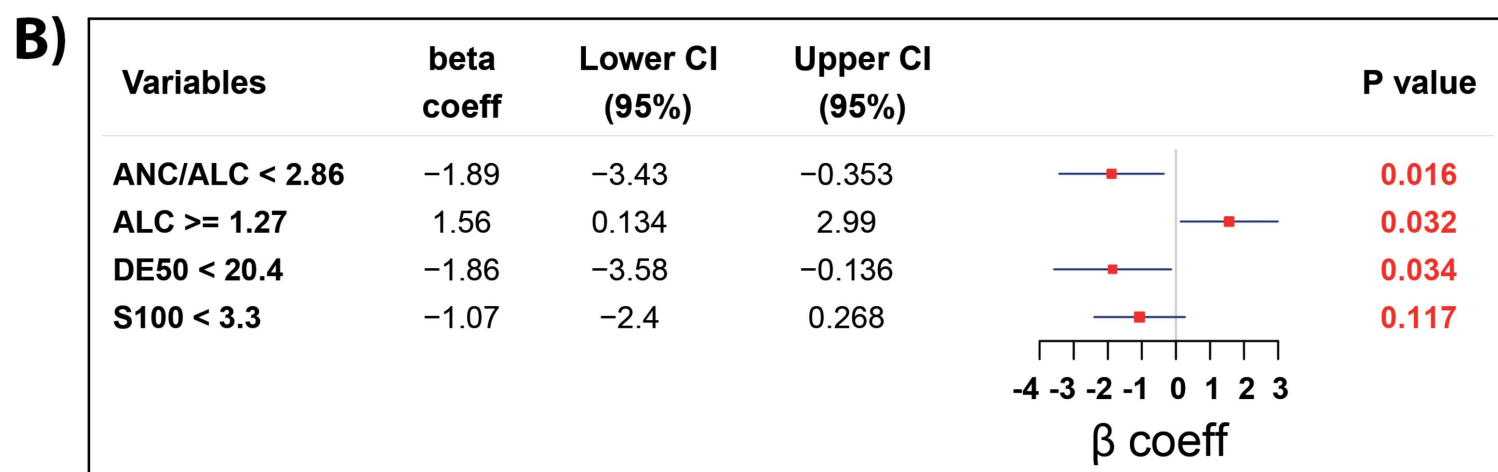
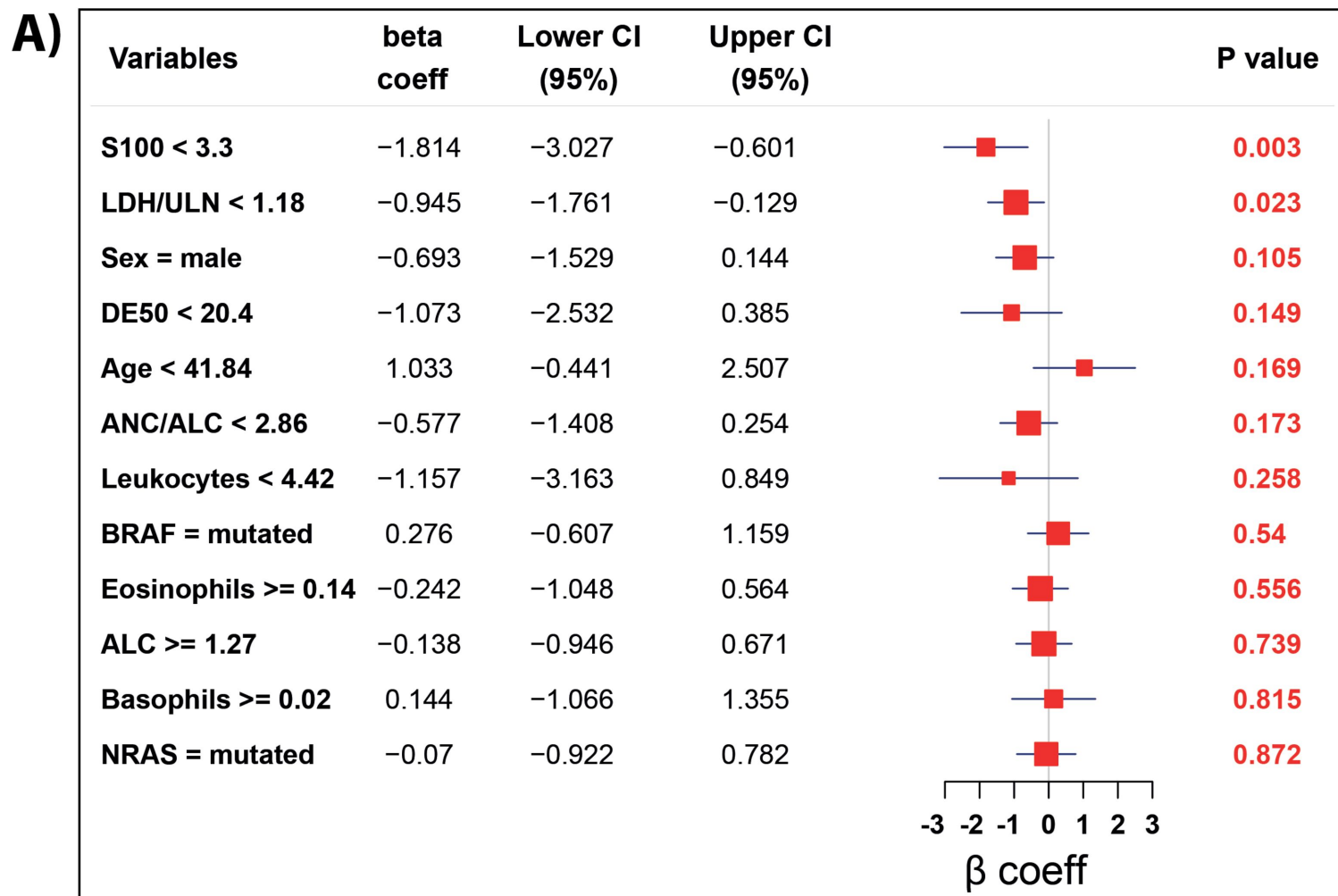


Figure 5

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